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STANDARDIZATION OF INSECTICIDES AND DISINFECTANTS.

By L. E. SAYRE.

AT the last annual meeting of this Academy the writer presented a brief review of the commercial insecticides, and referred to federal regulation No. 16, which requires certain ingredients of these compounds to be declared. Prominent among the constituents affected by this regulation is that of arsenic and any of its combinations.

Inasmuch as this class of agents, and those closely related, the bactericides (disinfectants), have come under the regulation affecting standards, I have thought it wise to present to this Academy in compact form the present legal status, so to speak, of these two classes of agents, so that we shall have for reference in our report of proceedings reliable data concerning them. In order to do this I may have to revise and add to what was stated on this subject in a paper presented to this Academy last year.

In the first place, it should be stated regarding insecticides, that insecticides other than arsenical combinations and fungicides containing inert substances which do not prevent, destroy, repel or mitigate insects or fungi, must bear a statement on the label of the name and percentage of each inert substance therein, unless the name and percentage of each active ingredient of the article is plainly and correctly stated, in which case it will be sufficient to state on the label that the article contains inert substances, giving correct percentage thereof.

The enactment of the federal law has naturally drawn much attention to the subject of the relative value of fumigants, sprays and powders acting as insecticides, and recent investigation of these in our own laboratory has shown that our opinions must be revised as to relative toxicity of members of this class. For example, it was believed at one time that formaldehyde ranked in the first class among these agents. Actual experiments, however, indicate that it is quite low in activity; for instance, it has one-tenth the power of sulphur dioxide.

For our work on insecticides, "Bell jar experiments" were carried out, such as described by Hamilton and Lowe.¹ This apparatus is so constructed as to make possible the measuring of

1. See *Journal of the American Public Health Association*, 1911.

definite amounts of gases, which may be drawn from a container for any experiment. Such gases as illuminating gas, sulphur dioxide, carbon dioxide, hydrogen sulphide, etc., have been employed. Insects confined within the chamber of the apparatus and subjected to the influence of various insecticides can be watched and timed so that the relative value of toxic action can be readily estimated.

Hamilton and Lowe have tabulated in their report the coefficients for thirty-eight different insecticides, including such articles as creosote, naphthalene, kerosene, oil of turpentine, hydrocyanic acid, and many powders.

Our own experiments have shown that a solution of carbolic acid, from 6 to 7 per cent, acts very powerfully by the contact method, but much less powerfully as a fumigant; it has $\frac{1}{50}$ the strength of sulphur vapor, for example. It may be said of many of the commercial plant powders that they are exceedingly weak as compared with sulphur. A powder known as "bugbane" (*Cimicifuga racemosa*) has long been considered efficacious. Our experiments with it, however, tend to show that this drug has been greatly overestimated in its toxic properties toward insects. Powdered cimicifuga seemed to be devoid of insecticidal properties. Crickets kept in contact with the powdered drug for hours showed no toxic effect.

For the chemical valuation of the well-known insect powder, H. Linke has suggested a method. (See Chem. Abstracts, vol. 6, No. 23, p. 3493.)

The following articles compounded with insecticides require to be guaranteed under the national insecticide act, when sold for insecticidal purposes:

Acid, carbolic, cryst.	Insect powder, foreign.
Acid, carbolic, crude.	Naphthalene.
Acid, cresylic.	Nitrobenzene.
Acid, muriatic.	Oil citronella.
Acid, nitric.	Oil camphor.
Acid, sulphuric.	Oil peppermint.
Ammonia, stronger.	Paris green.
Arsenic, white.	Phosphorus.
Borax.	Potassium cyanide.
Camphor.	Potassium permang.
Carbon bisulphide.	Potassium carb.
Carbon tetrachloride.	Potassium sulphide.
Copper sulphate.	Quassia.
Formaldehyde, sol. of.	Rosin.
Hellebore, white.	Sulphur, grd. or lump.
Iron sulphide.	Sulphur, refined.
Lead arsenate.	Turpentine.
London purple.	Whale oil soap.
Iron sulphate.	

DISINFECTANTS.

Having been appointed as one of the members of a committee to report on standards for disinfectants, it may be in place to call attention to the ruling of the Kansas Board of Health, which was based on the report of the committee. The tentative standard adopted by this board reads as follows:

A substance may be said to be a disinfectant or germicide, or to act as a germicide, when under stated conditions of concentration, temperature, humidity, etc., it is able to kill any non-spore-bearing bacterium pathogenic to man within six hours. Unless otherwise expressly stated, temperature and other atmospheric conditions usually found in living rooms will be understood.

Within the meaning of this definition the terms "germicide" and "disinfectant" are used interchangeably to mean substances that actually destroy, and not merely inhibit the growth of bacteria.

Various methods have been used for standardization of disinfectants, beginning with Pringle (1732), who attempted to arrest putrefaction by the addition of various substances, and including Kock's "thread method" (1881), which was the first systematic test for the germicidal power of disinfectants. Kock's method was followed by the Rideal and Walker or "drop method" (1903) and its various modifications.

After consideration of all these methods of different workers, the committee on standardization of disinfectants recommended for adoption as the standard methods the Hygienic Laboratory phenol coefficient methods, as devised by Doctors Anderson and McClintic and published in Hygienic Laboratory Bulletin No. 82.

To secure uniformity, the third distillate of Merck's Silver Label Phenol was used in making the 5 per cent stock dilutions, from which all other phenol dilutions were made.

To obtain a culture of even resistance, it was recommended to use *B. typhosus* from a 30-day at 20° C. stock culture on standard infusion agar. Transplants are made at intervals of twenty-four hours in usual manner. The procedure is, briefly, as follows:

Since most disinfectants have a coefficient of 1 or over, a stock dilution of 1-100 is made of the disinfectant, but for those having a coefficient less than one a 1-20 is used. These dilutions are measured by means of pipettes into sterile glass-stoppered graduated cylinders containing sterile distilled water, and made up to the mark with same. From these stock dilutions the necessary dilutions for tests are made, using sterile pipettes of several sizes, and adding the required amount of the stock dilution and distilled water from sterile lead-foil-topped flasks to lead-foil-topped sterile bottles.

The phenol dilutions are tested first, beginning with the strongest, and then taking up the disinfectants in the same order.

To each sterile seeding tube 5 cc. of the dilutions are added and inoculated with $\frac{1}{10}$ cc. of filtered culture from 24-hour *B. typhosus* by means of a sterile pipette graduated in tenths.

The inoculated seeding tubes are placed on water bath and kept at temperature of 20° for lengths of time $2\frac{1}{2}$ minutes to 15 minutes, making six tests in as many seeding tubes for each dilution.

The sterile subculture tubes of same number as dilutions, and 4 mm. platinum loops (4 in number) and fan-tail burner must be conveniently arranged for inoculation of subculture tubes and flaming of loops. These inoculations are made at intervals of $2\frac{1}{2}$ minutes until six inoculations have been made, at times $2\frac{1}{2}$ minutes, 5 minutes, $7\frac{1}{2}$ minutes, 10 minutes, $12\frac{1}{2}$ minutes and 15 minutes for each dilution.

The coefficient for the sample under investigation is obtained by dividing the weakest dilution of this sample of disinfectant that kills in $2\frac{1}{2}$ minutes by the weakest dilution of phenol that kills in $2\frac{1}{2}$ minutes, and dividing the weakest dilution of the above sample of disinfectant that kills in 15 minutes by the weakest dilution of phenol that kills in the same time; then taking the mean of these quotients.

For example, the weakest dilution of phenol proving effective in $2\frac{1}{2}$ minutes was 1-80 and the weakest dilution of the above disinfectant proving effective in $2\frac{1}{2}$ minutes was 1-375. The weakest phenol dilution proving effective in 15 minutes was 1-110 and the weakest dilution of the sample of disinfectant was 1-650; hence, $\frac{375}{80} = 4.69$ and $\frac{650}{110} = 5.91$; $5.91 + 4.69 = 10.60$. $\frac{10.60}{2} = 5.30$, the phenol coefficient for the sample of disinfectant under investigation. Its standard, therefore, would be 5.30; that is, 5.30 times as strong as a solution of phenol that kills in the same length of time.